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On the morphology of the connective tissue of ixodid ticks

by

V. A. Tsvileneva

The connective-tissue elements in ixodid ticks are represented by the hemolymph and loose connective tissue with its condensations around the internal organs, such as the intramuscular septa, the sarcolemma, the connective-tissue stroma of the ovaries, the condensation of the connective tissue around the organs of the digestive system, on the malphigian vessels, the basal membranes, and so forth. In the literature on the histology of ixodid ticks, the greatest attention has been given to the formed elements of their hemolymph. Studies by the author of the present paper have also been devoted to this same subject (Tsvileneva, 1959, 1961a, Muratov and Tsvileneva, 1960). With regard to the other connective-tissue formations, on the other hand, we have found only an occasional statement to the effect that they are poorly represented in ixodid ticks (Samson, 1909, Balashov, 1961).

The objects of our study have been adult female ixodid ticks of the species Hyalomma detritum, Boophilus calcaratus, and Rhipicephalus sanguineus in various physiological states.

The connective tissue was studied in sections through ticks embedded in celloidin (fixation in Zenker's fluid with formalin, staining with hematoxylin-eosin, azure-eosin, azan) and also in connective-tissue films [tr.: layers?] fixed in formalin, Zenker's fluid with formalin, and stained with hematoxylin-eosin, azure-eosin, azan, and

Heidenhain's and Yasvoin's [tr.: sp.?] iron hematoxylin, impregnated by the Bil'shovskii technique, and stained with orcein by the Tanzer-Unna method.

The connective-tissue formations are most highly developed in satiated females. The connective tissue of such females has been taken as study material for the present paper.

Female ixodid ticks are rich in connective-tissue cells. A considerable part of their body cavity, free of internal organs, is filled with interlaced and anastomosing layers of amorphous substance of loose connective tissue. Large lacunae filled with hemolymph lie free among them. In addition to this, there are numerous large and small apertures in the layers and strands of amorphous substance themselves, so that the hemolymph and the free cells suspended in it can readily circulate in the body cavity. On the surface of the organs, the amorphous substance is condensed and apparently takes part in the formation of boundary membranes; the sarcolemma, the basal membranes, and so on.

A large number of cells are contained in the meshes of the strands and membranes of the amorphous substance of the loose connective tissue. In the first place, the anastomoses of amorphous substance always bear a considerable number of hemocytes that have settled out of the channel. In the second place, cells of the desmoblastic series at various stages of differentiation -- from young desmoblasts to dead nuclei of desmocytes used up in the formation of ground substance -- are sealed into the amorphous substance. The source of the new desmoblasts in the adult tick is the fat body, and specifically, its undifferentiated basophil cells (Tsvileneva, 1961b). In film preparations, which always contain not only connective tissue but also cords of the fat body, it is easy to trace the separation of individual basophil cells of the fat body or small groups of them (Fig. 1). These groups of cells, situated on the amorphous substance, are spread out on it with their cytoplasm, which forms a thin layer. It is apparently at the cell boundaries that the formation of the ground substance takes place, as has been described for invertebrates by A. A. Zavarzin (1953), F. M. Lazarenko (1925), and others. In such small islands of isolated basophil cells, the marginal cells are soon seen to be isolated from the islands by the growing cytoplasm and the ground substance that is being formed. In these cells and some occasional desmoblasts, we can still see clearly the territory of the endoplasm around the nuclei, on the periphery exhibiting a transition into

ectoplasm and ground substance. Both in preparations stained with iron hematoxylin and azan and in preparations impregnated with silver, we can see clearly that the cells are sealed in the ground substance that surrounds them. Finally, there are also scattered nuclei here and there, sealed in the ground substance, devoid of any endoplasm and undergoing degeneration.

In connection with the fact that entire small segments of strands of fat body are regularly evolved in the formation of the ground substance, it would appear that the enocytes (Tsvileneva, 1961б), which are situated on the fat body and seem to be incapable of independent movement, sometimes place themselves not only in the strands of the fat body but also close the groups of separating desmoblasts, directly on the films of amorphous substance. Thus, the enocytes also sometimes appear as cells that are seated directly on the anastomoses of the ground substance of the connective tissue.

We were unable, using either the silver method or azan or orcein staining, to discover in the loose connective tissue any specific fibrillar elements of the type of collagen fibers or elastic fibers, as had been reported by Ochse (1946), Baccetti (1956), and others in insects. It is also possible that such fibrous elements are not present in ticks, since their loose connective tissue shows a peculiar substitution: evidently in connection with an adaptation to sharp changes in form and volume, the tick's body is found to contain not only somatic muscles but also many visceral muscles of highly varied appearance. Their fibers run through the ground substance in various directions. The transverse striations of these fibers are particularly evident with silver impregnation and azan staining of films of loose connective tissue. In addition to a considerable number of relatively large bundles of fibers, the amorphous substance contains a large number of long, abundantly branching, slender bundles of fibers (Fig. 2), occasional individual fibers, small bundles that pass into broad slender films with distinct and pronounced striation (Fig. 3). Branching muscle fibers, closely bound to the amorphous substance, along which they run, again separate from such films in various directions. Some of these fibers proceed from connective tissue on the walls of the organs. There the visceral muscles are already disposed in regular layers.

While the relatively large somatic muscles of the tick have a prominent sarcolemma, large accumulations of sarcoplasm under it, and numerous muscle nuclei disposed along the periphery of the fibers, the visceral muscles do not have a distinct membrane that would

separate them from the surrounding connective tissue. The cytoplasm is in slender, often solitary bundles passing through the ground substance and is appreciable only in the immediate vicinity of nuclei that have the characteristic elongated shape and are of various sizes depending on the thickness of the bundle. The nuclei have 1 or 2 nucleoli. At the points where the muscle fibers branch, there is a considerable amount of cytoplasm, the nuclei are scattered densely and are usually rounded. In transversely striated films, the nuclei are rounded and markedly compressed. However, even such flat nuclei, 2-3 micra thick, are thicker than the films themselves to which they belong. In some occasional transversely striated fibers, the nuclei are very small.

It is thought that Morison (1928), in his detailed work on the muscles of the bee, erroneously described the sarcolemma as belonging to the visceral muscles. To judge from the drawings reproduced by Morison, it is not present in the visceral muscles of insects as it is in ticks, and the author has purely mechanically transferred this representation of the structure of the muscle fiber of the visceral muscles from the morphology of the somatic muscles of the same object, of which he has given such a good analysis.

In spite of the fact that the visceral muscles of the ixodid ticks have transverse striations (like the visceral muscles of all arthropods) and a syncytial structure, like the somatic muscles, they differ greatly from the somatic muscles in their structure, which characterizes them as connective-tissue elements: they are not demarcated from the surrounding connective tissue by a sarcolemma; on the contrary, they are closely bound to the amorphous substance of the connective tissues, and in some places, transforming themselves into thin layers, assume a still closer similarity to the substrate along which they proceed and with which they have a close genetic bond. Finally, it is probable that in this case they also serve functionally not only as purely muscular structures but also as the mechanical fibrous elements of the loose connective tissue. The above-described network of contractile elements of the internal medium of the tick excludes the need for any other mechanical elements in its ground substance.

Conclusions

A statement has appeared very recently to the effect that ixodid ticks do not have a fat body and that the only place of storage of reserve substances in the tick is the cells of its

intestinal epithelium (Balashov, 1961). Iu. S. Balashov, having earlier published a detailed study of the physiology of intracellular digestion in ixodids (1957), described an extremely interesting phenomenon: the utilization of the intestinal epithelium as a place of accumulation of reserve fats and glycogen. The author speaks in the same place of reserve proteins. It would appear, however, that the term "reserve proteins", strictly speaking, should not be used to designate the alimentary inclusion bodies that are found in the tick, containing the host's blood at various stages of digestion. Together with the contents of the intestinal cavity, this, of course, is the reserve that is used by the tick to build the live substance of its body and to synthesize the reserve fats and glycogen grains that are formed from the sugar likewise contained in the host's blood. But these alimentary inclusions, of course, do not represent the proteins of the tick's body and cannot be called its reserve proteins, aside from the fact that the author himself did not discover even one protein in these alimentary reserves. Indeed, the tick does have its own reserve proteins, but these are to be found precisely in the fat body (Tsvileneva, 1961б). the fat body in the ixodid ticks has also been described by E. K. Suvorov (1908) and then in Samson's studies (1909) and in those of Nordenskjold and others. The absence of any anatomical separation of the fat body has apparently also led Balashov to deny its existence in the ixodids. We already have had occasion to point out, however, that both functionally and genetically the fat body is entirely comparable to the fat body in insects (Tsvileneva, 1961a, 1961б). The absence of anatomical peculiarities in the form of massive lobes or lubules appears to be characteristic of the arachnids and certain other arthropods. Thus, Millo (1926, 1949), describing the "interstitial tissue" (tissu interstitiel) of spiders, emphasizes its analogy and homology to the fat body of the insect. Debaisieux (1953), working with females of Argulus foliaceus (Crustacea), have described the "accumulation tissue" (tissu d'accumulation) as also functionally and genetically equivalent to the fat body in other arthropods. The fact that Balashov failed to perceive the fat body in the tick also explains his erroneous statement in the aforesaid paper that the sole place of storage of RNA in the tick is the oocyte. The basophilic cells of the fat body, characterized by their large RNA content, represent the cambium of the loose connective tissue and of the fat body itself, and perhaps also synthesize a protein component for reserve inclusion bodies of a glycoprotein nature, stored in the cells of the fat body.

The hitherto widespread opinion among zoologists that arthropods have a very scanty connective-tissue supply is disputed in some new studies on insects (Pipa and Cook, 1958). In some insects, the connective tissue fills the entire body cavity, surrounding and

consolidating the organs, so that it becomes impossible to speak of an open blood channel. This is also true of ixodid ticks, the loose connective tissue of ixodid ticks is rich in a variety of cellular elements, both migrating and stationary. It contains strands of fat body closely joined to it, representing a variety of connective tissue, and fibers and layers of visceral muscles run through the connective tissue. The hemolymph circulates largely through the communicating large and small lacunae in the loose connective tissue, which, with the hemolymph, fills the tick's mixed body cavity.

(The summary on page 177 of text is in Tadzhik).

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Figure 1

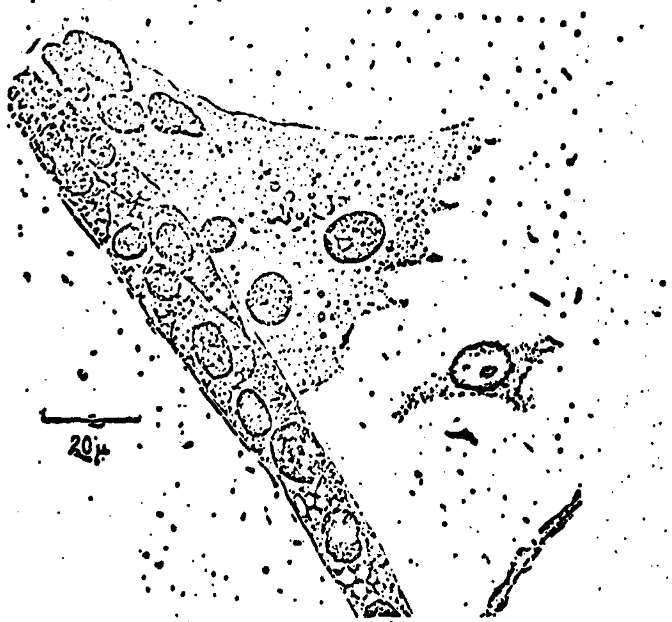
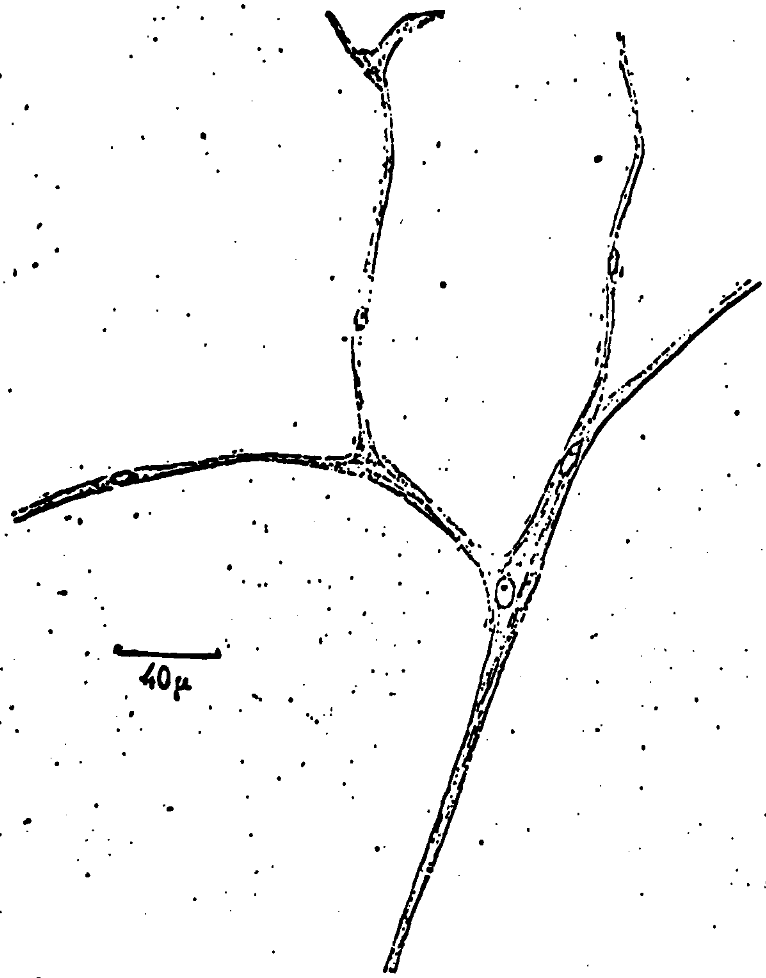


Рис. 1. Пленочный препарат соединительной ткани Boophilus calcaratus. Фиксация по Максимову, окраска азаном. Окуляр ×15, объектив ×50, имм. На пленке аморфного вещества лежит тяж жирового тела и обособляющиеся от него десмобласты.

Film [tr.: impression?] preparation of connective tissue of Boophilus calcaratus. Fixation according to Maksimov, staining with azan. Ocular X15, objective X50, imm. On the film of amorphous substance lies a strand of the fat body and desmoblasts that are separating from it.

Figure 2



Film preparation of connective tissue of Boophilus calcaratus. Fixation according to Maksimov, staining according to Yasvoin. Ocular X15, objective X20. A visceral muscle fiber branching into loose connective tissue of tick.

Рис. 2. Пленочный препарат соединительный ткани Boophilus calcaratus. Фиксация по Максимову, окраска по Ясвоину. Окуляр ×15, объектив ×20 Волокно внутренностных мышц, ветвящееся в рыхлой соединительной ткани клеща.

Figure 3

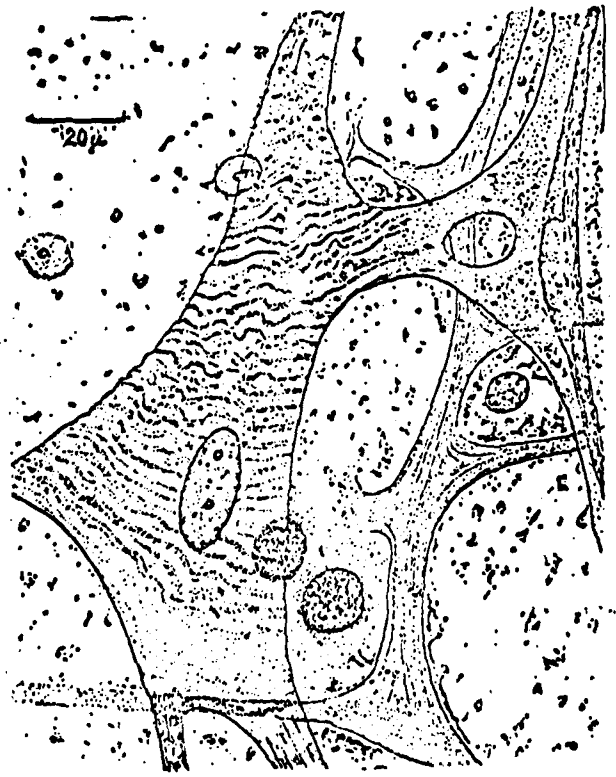


Рис. 3. Пленочный препарат соединительной ткани Boophilus calcaratus. Фиксация по Максимову, окраска азаном. Окуляр ×15, объектив ×50, имм. На пленке аморфного вещества располагаются в 2 слоя разветвляющиеся мышечные пленки, переходящие в волокна. В аморфном веществе видны ядра клеток десмобластического ряда разной степени дифференцированности.

Film preparation of connective tissue of Boophilus calcaratus. Fixation according to Maksimov, staining with azan. Ocular X15, objective X50. imm. On the film of amorphous substance there are 2 layers of branching muscle films, showing a transition into fibers. In the amorphous substance we see nuclei of cells of the desmoblast series of various degrees of differentiation.

PDeporte, translator, 12/3/65